F1-029

UNIVERSITY OF CALIFORNIA, DAVIS

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JUL 22 1997

CALFED Bay-Delta Program Office
1416 Ninth Street, Suite 1155
Sacramento CA 95814

Research Proposal Entitled
"Developing Genetic Markers to Assist Monitoring Programs in Distinguishing Between Reithrodontomys raviventris and R. megalotis..."
RFP: 1997 Category III Ecosystem Restoration Projects and Programs
Principal Investigator - **Michael L. Johnson**

Dear Colleague:

It is our pleasure to present for your consideration the referenced proposal in response to **the CALFED Bay-Delta Program RFP.**

Please call on the principal investigator for scientific information. Administrative questions may be directed to me or my assistant, René Domino, at the above address and phone number. We request that correspondence pertaining to this proposal and a subsequent award be sent to the Office of Research and to the principal investigator.

Sincerely,

Sandra M. Dowdy
Contracts and Grants Analyst

Enclosure

cc: M. Johnson

I-000873

Executive Summary

Title: Developing Genetic Markers to Assist Monitoring Programs in Distinguishing Between *Reithrodontomys raviventris* and *R. megalotis*, with the Development of a Demographic Index to Assess the Status of *R. raviventris* Populations

Michael L. Johnson, Principal Investigator, Bernie P. May, Co-Investigator; University of California, Davis

Project Description

The purpose of this project is to develop genetic markers to distinguish salt marsh harvest mice (*Reithrodontomys raviventris*) and western harvest mice (*Reithrodontomys megalotis*), and to develop a demographic index to monitor the viability of populations of salt marsh harvest mice. The results of this project can be used in support of monitoring programs following the restoration or enhancement of wetlands throughout the North Bay, Suisun Bay, and Delta regions, and in monitoring programs already in place in the Bay-Delta system.

The specific objectives for this project are: 1) Sample the entire northern range of both species to collect hair and/or cheek cell samples from salt marsh harvest mice and western harvest mice in as many habitats as possible. 2) Using Amplified Fragment Length Polymorphisms (AFLP), develop a marker(s) to reliably distinguish between the two species. 3) Assess the amount of genetic variation throughout the North Bay, Suisun Bay, and Delta in the two species. 4) Determine if the northern populations are subdivided into isolated subpopulations, e.g., a western form separated from an eastern form by the Napa River. 5) Determine if any introgression is occurring between salt marsh harvest mice and western harvest mice at any point along the interface of the two species. 6) Compare the demographic characteristics of the individuals captured at each location with the same characteristics of animals from established populations of salt marsh harvest mice to evaluate the potential viability of populations in the sample locations. 7) Evaluate samples submitted by the resources agencies to provide species identifications for monitoring projects.

Approach

The approach involves live trapping individuals of both species and removing either a few hairs or cells from the lining of the cheek. These sampling techniques are noninvasive and require no disruption of an individual's life history. Genetic markers will be developed using the Amplified Fragment Length Polymorphism technique. After the markers are developed, they will be available to verify any monitoring efforts by any agency trapping mice in the Bay-Delta system. In addition, by comparing to the demographic performance of known viable salt marsh harvest mouse populations (data are currently being analyzed), all populations sampled during the course of this project will be characterized as to their viability status. Demographic characterization will be determined by a multivariate statistical approach.

Justification for Project

The species targeted in this proposal is the salt marsh harvest mouse, not a priority species identified within the CALFED Implementation Strategy. However, we believe that this project is in keeping with the CALFED mission because restoration activities that will benefit the species

identified as priorities to the CALFED will also impact (both positively and negatively) the salt marsh harvest mouse, a federally and state listed endangered species. Our project will insure that any activities targeted to restore CALFED priority species can be evaluated in light of their potential impacts on harvest mice. Our project will provide the baseline genetic information to identify salt marsh harvest mice, assess the levels of genetic variability in the population, determine population structuring, evaluate the effects of any restoration activities on the genetic structure of the populations. By developing a demographic index of population viability, a general determination of the status of *R. raviventris* populations in the North Bay and Suisun Bay can be made. Additionally, under certain conditions salt marsh harvest mice can occupy seasonal wetlands and saline emergent wetlands, priority habitats under the CALFED mission. Supporting a project targeting the salt marsh harvest mouse guarantees a true ecosystem approach to restoration and management efforts within the Bay-Delta system. Finally, the monitoring system developed during this project will be useful to CALFED agencies in their monitoring programs that fall outside of the CALFED mission.

Budget Costs and Third Party Impacts
Total Cost: $250,243
There will be no negative third party impacts as a result of this project. The project is designed to allow resource agencies to submit samples for analysis during the project.

Applicant Qualifications
Dr. Michael Johnson is a Research Engineer at the University of California at Davis who has been studying the ecology of the salt marsh harvest mouse in the North Bay for the last 3 years. He has published numerous papers on the ecology and demography of small mammals. He holds the necessary federal and state permits to work with the salt marsh harvest mouse. Dr. Bernie May is a Research Scientist at the University of California Davis who has been conducting genetic analyses of endangered species for several years. He has published extensively on the genetic structure of animal populations. He maintains a molecular genetics laboratory with state-of-the-art equipment. The techniques used in this investigation are noninvasive and result in no injury to any animals.

Monitoring and Data Evaluation
The results of this project will be submitted for publication in peer reviewed journals. All data will be available to CALFED agencies.

Compatibility with CALFED Objectives
By realizing our objectives, we will develop the framework that will provide information to CALFED about the success of restoration projects targeting salt marsh harvest mice, and provide a framework that can evaluate the impact of projects that indirectly negatively affect salt marsh harvest mice. This project will improve the ability of the resources agencies to manage one of the endangered species within the estuary.

Developing Genetic Markers to Assist Monitoring Programs in Distinguishing Between *Reithrodontomys raviventris* and *R. megalotis*, with the Development of a Demographic Index to Assess the Status of *R. raviventris* Populations

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Type of Organization: State Agency (University)
94-6036494-W
Financial Contact Person: Office of Research (916-752-2075/5432 fax)

RFP Project Group Type: Other Services

Principal Investigator: Michael L. Johnson

Typed Name of Authorized Representative:

Signature of Authorized Representative: Sandra M. Dowdy

Title: Sandra M. Dowdy
Telephone number: Contracts and Grants Analyst (916) 752-2075
Date Signed: JUL 22 1997

I-000876

Project Description and Approach

The purpose of this project is to develop genetic markers to distinguish salt marsh harvest mice (SMHM, *Reithrodontomys raviventris*) and western harvest mice (WHM, *Reithrodontomys megalotis*), and to develop a demographic index to monitor the viability of populations of SMHM. Currently, the two species are almost indistinguishable morphologically. Ecologically, they are very similar with SMHM inhabiting the pickleweed marshes of the San Francisco Bay-Delta region, and the western harvest mouse inhabiting the uplands adjoining these marshes. At the interface of these two habitats and in marginal habitat, the two species can be sympatric. Restoration of wetlands often involves disturbance of the habitat and may require long periods of time before habitat would be considered optimal for SMHM. Consequently, it is not possible to easily and accurately evaluate the effects of activities aimed at restoring SMHM populations, or restoration efforts focused on other species that may secondarily impact the viability of SMHM populations. The results of this project can be used in support of monitoring programs following the restoration or enhancement of wetlands throughout the North Bay, Suisun Bay, and Delta regions, and in monitoring programs already in place in the Bay-Delta system. The approach involves live trapping individuals of both species and removing either a few hairs or cells from the lining of the cheek. These sampling techniques are noninvasive and require no disruption of an individual's life history. Genetic markers will be developed using Amplified Fragment Length Polymorphism technique. After the markers are developed, they will be available to verify any monitoring efforts by any agency trapping mice in the Bay-Delta system. In addition, by comparing to the demographic performance of known viable SMHM populations, all populations sampled during the course of this project will be characterized as to their status. Demographic characterization will be determined by a multivariate statistical approach.

Location of Project

The project will be carried out in the North Bay, Suisun Bay, and Delta region, roughly the northern extent of the geographic range of the salt marsh harvest mouse. We will trap as far east into the Delta as necessary to sample salt marsh harvest mice and western harvest mice. This range includes the counties of Marin, Sonoma, Napa, Solano, and Contra Costa, and potentially the counties of Sacramento and San Joaquin.

Expected Benefits

The species targeted in this proposal is the salt marsh harvest mouse, not a priority species identified within the CALFED Implementation Strategy. However, we believe that this project is in keeping with the CALFED mission because restoration activities that will benefit the species identified as priorities to the CALFED will also impact (both positively and negatively) the salt marsh harvest mouse, a federally and state listed endangered species. Our project will insure that any activities targeted to restore CALFED priority species can be evaluated in light of their potential impacts on harvest mice. Our project will provide the baseline genetic information to identify salt marsh harvest mice, assess the levels of genetic variability in the population, determine population structuring, and evaluate the effects of any restoration activities on the genetic structure of the populations by developing a demographic index of population viability. Additionally, SMHM do occupy seasonal wetlands and saline emergent wetlands (Koerner 1997, Koerner and Johnson in prep), priority habitats under the CALFED mission. Supporting a project targeting the salt marsh harvest mouse guarantees a true ecosystem approach to restoration and

management efforts within the Bay-Delta system. Finally, the monitoring system developed during this project will be useful to CALFED agencies in their monitoring programs that fall outside of the CALFED mission. M. Johnson has been contacted by members of agencies (e.g., Department of Water Resources, California Department of Fish and Game) requesting help in distinguishing between these two species. A genetic marker available to these agencies would be invaluable in allowing them to quickly and confidently complete their monitoring.

Background and Biological Justification

One of the major features of the CALFED framework is the restoration of habitat in the North Bay, Suisun Bay, and the Delta. High in priority for restoration are wetlands, including tidal and seasonal freshwater wetlands. While specific fish species are targeted from these activities, a broad range of wildlife and fish species will benefit from these restoration activities including several endangered and threatened species. The salt marsh harvest mouse is one of those species that will benefit by the restoration of high tidal marsh dominated by pickleweed, and to a lesser extent, by the restoration of seasonal wetlands. Monitoring the success of the restoration efforts will require that populations of harvest mice be identified, monitored, and their demographic performance evaluated. The success or failure of some wetland restoration projects and the monitoring programs that accompany these projects could depend on reliably documenting the viability of populations of SMHM.

The first step of these monitoring efforts is to verify the species identity of the salt marsh harvest mice. Although this seems like a trivial exercise, such is not the case. The salt marsh harvest mouse is almost indistinguishable from the western harvest mouse. The taxonomic key is marginal in its ability to distinguish the two, except in the hands of mammalogists with considerable experience with the two species. Distinguishing between the two species is especially problematic in the North Bay and Suisun Bay marshes where the two species are morphologically very similar. Most investigators rely on a combination of features including pelage markings, behavior, and the habitat in which the individuals are found. While these may be reliable for many established populations in large stands of pure habitat (i.e., large tracts of pickleweed), in smaller marshes and those in the process of being restored, reliable identification of SMHM and WHM is very difficult. We recently monitored populations of SMHM in the Napa Marsh Unit of the San Pablo Bay National Wildlife Refuge, a seasonal wetlands. Only after trapping, marking, and monitoring individuals over long periods of time were we confident of our identifications. Most monitoring programs do not have a similar amount of time and effort that can be devoted to identifying species. We propose to develop a genetic biomarker(s) that can reliably distinguish between the SMHM and the WHM throughout the entire North Bay, Suisun Bay, and as far east into the Delta as SMHM extend. We will be able to use either hair follicles or cells scraped from the inner cheek for DNA analysis making the entire sampling process noninvasive. Once the genetic marker(s) is developed, we will work with the agencies responsible for monitoring SMHM populations to identify samples submitted to our laboratory. Any entity associated with monitoring SMHM populations will be able to submit hair or cheek cell samples for verification that the species is identified correctly.

Verifying the presence of SMHM in a marsh does not guarantee that the population is viable (defined as self-sustaining for some unspecified time into the future). Individuals may be

transients in the area as we recently discovered in a small, degraded wetland on Mare Island. Alternatively, they may be resident, but the habitat is suboptimal and the population exists only because animals immigrate from viable, source populations nearby. These sink habitats may actually maintain population numbers as high as source populations, but the key is that the population growth rate is below 1.0 (the growth rate at which the population remains stable). It may be possible to detect which populations are sources and sinks because the sink populations would be composed primarily of individuals migrating from elsewhere. Immigrating individuals are usually demographically distinct from resident populations in that dispersing individuals are more often subadults and predominantly male (*Johnson and Gaines 1988, 1990*). We recently finished a two year demographic study of a viable SMHM population in the San Pablo Bay National Wildlife Refuge. Using the demographic characteristics from that population, we will develop a demographic index of a viable population. We will then be able to compare this index with indices developed from populations we sample for the genetic analyses. Comparisons will enable us to determine if our sample populations are source or sinks.

The specific objectives for this project are:
1) Sample the entire northern range of both species to collect hair and/or cheek cell samples from SMHM and WHM in as many habitats as possible.
2) Using Amplified Fragment Length Polymorphisms (AFLP), develop a marker(s) to reliably distinguish between the two species.
3) Assess the amount of genetic variation throughout the North Bay, Suisun Bay, and Delta in the two species.
4) Determine if the northern populations are subdivided into isolated subpopulations, e.g., a western form separated from an eastern form by the Napa River.
5) Determine if any introgression is occurring between the SMHM and the WHM at any point along the interface of the two species.
6) Compare the demographic characteristics of the individuals captured at each location with the same characteristics of animals from established populations of SMHM to evaluate the potential viability of populations in the sample locations.
7) Evaluate samples submitted by the resources agencies to provide species identifications for monitoring projects.

By realizing these objectives, we will be able to provide information to CALFED about the success of restoration projects that would have SMHM as a potential target, and provide information about the genetic structure of the SMHM population in the North Bay, Suisun Bay, and Delta. Because the genetic structure of the population is one of the most critical pieces of information necessary for any species that requires management (Baverstock and Moritz 1996), this project will improve the ability of the resources agencies to manage one of the endangered species within the estuary.

Proposed Scope of Work

Salt Marsh Harvest Mouse and Western Harvest Mouse Biology

The salt marsh harvest mouse is a state and federally listed endangered species endemic to the salt marshes of the Bay. Shellhammer et al. (1982) identified optimal habitat for *R. raviventris* as tall dense pickleweed, intermixed with fat hen (*Atriplex patula*) and alkali heath, with considerable

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stratification of the canopy. During flood tides, mice can avoid the water by moving up into the vegetation, moving to higher non-flooded areas within the marsh, or moving to adjacent uplands (Dixon 1908, Fisler 1965, Shellhammer 1989). Habitation of upland areas or seasonal wetlands may occur for extended periods of time if the marshes remain flooded (Koerner 1997, Koerner and Johnson in prep.). If the occupation of the uplands lasts for more than a few hours or days, the salt marsh harvest mice may come into contact with western harvest mice which normally occupy grasslands (Pitcher and Keller 1979, Heske et al. 1984, Johnson and Gaines 1988).

Sampling Protocol

As many sample locations as possible will be established in the North Bay, Suisun Bay, and the Delta. Exact locations will be determined later, but will include known established populations, locations at which difficulty in distinguishing between the two species has been encountered, locations that have been restored to wetland status, and locations that are targeted for future restoration. Using these criteria, during the first year of the project, a wide range of geographic locations and a variety of habitats will be selected. During the second year, additional locations within the range will be selected to assure that the most complete sample possible is acquired. These locations will include as many habitat types as possible, from pure pickleweed marshes to upland areas of annual grassland/shrubland. As many traps as possible will be placed at each location to ensure the entire site will be sampled for mice. Traps will be standard Sherman live traps baited with a mixture of birdseed and walnuts, and provided with cotton as nesting material. M. Johnson holds federal and state permits to trap SMHM. According to permitting restrictions, during the cool season pickleweed will be added to the traps, and traps will be wrapped in plastic bags secured with rubber bands to ensure that no animals are exposed to the rain while inside the traps.

At each capture, we will record: location, putative species, sex, reproductive condition, and body mass. Location will be recorded with a Global Positioning System unit which will be differentially corrected to obtain an exact location for each individual. Sex, reproductive condition, and body mass will be used to develop an index of population condition which will be compared with similar data from established populations that we have been monitoring for the past two years. (We are currently analyzing demographic data from a two year study of SMHM populations from this area.) For example, established (source) populations typically have large numbers of reproductively active adults of both sexes, while sink populations are more typically made up of predominantly nonreproductive subadult males. These areas are usually home to dispersing individuals that may find marginal habitat to occupy (Johnson and Gaines 1990). Reproductive condition will be determined by external characters. Males will be considered sexually active if they possess scrotal testes, females are reproductively active if they possess medium or large teats (indicative of lactation) or are visibly pregnant. Finally, at each capture, a small clip of overhair on the dorsal surface will be made using a small hair trimmer. This mark will remain visible for the length of the trapping at the site, and will insure that we do not collect samples from the same individual more than once. It will also help us to estimate the population sizes at each location. The mark includes only the longer overhair, and does not reach the underhair next to the skin. This guarantees that the animal will not lose insulation from the mark and suffer from exposure. The hair is replaced with the next molt, which for small mammal species occurs twice per year. We have used this fur-clip technique with great success in the past in our surveys for harvest

mice. Trapping will be conducted at each site until as large a sample as possible is obtained. Our goal is to obtain 40 samples from each putative population.

At each capture, a few hairs will be removed from the mice using forceps. Also, using a blunt toothpick, cells from the lining of the inside of the cheek will be removed. Scraping cells from the lining of the cheek involves no invasive procedures. This technique requires that only a few centimeters of the toothpick be inserted along the lingual surface of the teeth. The toothpick is rotated to touch the cheek and a few cells are removed in the process. At this point, it is unclear which technique will provide the best sample, and we will use both until we decide which provides the greatest amount of DNA with which to work. Samples will be stored in individually labeled centrifuge tubes and returned to the laboratory for analysis.

Demographic Analysis

We are currently analyzing demographic data from an established population of SMHM and will be able to determine the characteristics of a viable, source population. Standard demographic parameters are being determined including sex ratio, age structure, reproductive performance, survival, population growth rate and density, and recruitment via reproduction. These analyses are being performed using a software package developed by Charles Krebs. This package has been used numerous times in the past by large numbers of investigators. Once the demographic data are analyzed, we will perform a Nonmetric Clustering and Association Analysis (NCAA) using several of these demographic parameters (e.g., sex ratio, age structure, reproductive condition, abundance) to establish the normal demographic "trajectory" of a population (Landis et al. 1996). In this way, we will be able to account for seasonal variation in demography and the effects of abundance on the demographic characteristics. This characterization process has been used in analyses of ecosystems under chemical stress and is demonstrated to effectively distinguish between stressed and unstressed populations. The technique will similarly distinguish between source and sink populations of SMHM.

Genetic Analysis

A variety of molecular markers are suitable for the discrimination of taxa at the species and subspecific level (e.g., allozymes, mtDNA, microsatellites, multilocus DNA fingerprinting, and numerous other procedures involving PCR amplification of specific nuclear sequences in coupling with heat denaturation or restriction digestion). In this study we are also interested in being able to detect hybridization and introgression between these two harvest mice and to develop a diagnostic molecular marker system whereby non-destructive samples from mice can be sent to us routinely by state and federal management biologists for identification. Thus, allozymes and mtDNA analyses will be unsuitable. Further, an analysis of mtDNA would probably not be effective since these two closely related taxa have not been isolated long enough to have accumulated fixed allelic differences (see related *Peromyscus* work by Avise et al. 1983). Dr. May's recent work on the endangered Northern Idaho ground squirrel (*Spermophilus brunneus*) with microsatellites (May et al. 1997, and unpublished population data) suggests that homoplasy at particular microsatellite loci can render such loci unsuitable for both population and species level comparisons. Recently we (and others) have shifted our focus to the use of amplified fragment length polymorphisms (AFLPs, Vos et al. 1995) to differentiate species, subspecies, and populations among tilapine and tui chub taxanomic groups (May et al., unpublished data). We

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will use restriction enzymes ECO RI and MSE I and specific primers (extension base pairs dependent on initial tests with both species DNA to optimize for numbers of intraspecific and interspecific variation) pre-labeled with fluorescent dyes. Detection of amplified products run on acrylamide gels will occur on a Molecular Dynamic 595 fluorimager. Variability for AFLPs will be scored as the presence/absence of particular bands. Taxa specific bands will be used to detect hybridization and introgression. Intraspecific bands will be used to assess levels of variation within and between populations. Frequencies of intraspecific bands that are not codominant will be calculated as the square root of the frequency of individuals in the population without the band. Intraspecific data will be analyzed with "Genes in Populations" (a computer program designed by B. May and C.C. Krueger and written in C by W. Eng and E. Paul).

Literature Cited

Avise, J.C., J.R. Shapira, S.W. Daniel, C.F. Aquadro, and R.A. Lansman. 1983. Mitochondrial DNA differentiation during the speciation process in *Peromyscus*. Mol. Biol. Evol. 1:38-56.

Baverstock, P. R. and C. Moritz. 1996. Project design. Pp. 17-27 *In*, Hillis, D. M., C. Moritz, and B. K. Mable, eds. Molecular Systematics. Sinauer Associates, MA.

Dixon, J. 1908. A new harvest mouse from the salt marshes of San Francisco Bay, California. Proc. Biol. Soc. Wash. 21:197-198.

Fisler, G. F. 1965. Adaptations and speciation in harvest mice of the marshes of San Francisco Bay. Univ. of Calif. Publ. Zool. 77:1-108.

Heske, E. J., R. S. Ostfeld, and W. Z. Lidicker, Jr. 1984. Competitive interactions between *Microtus californicus* and *Reithrodontomys megalotis* during two peaks of *Microtus* abundance. J. Mamm. 65:271-280.

Johnson, M. L. and M. S. Gaines. 1988. Demography of the western harvest mouse, *Reithrodontomys megalotis*, in eastern Kansas. Oecologia 75:405-411.

Johnson, M. L. and M. S. Gaines. 1990. Evolution of dispersal: theoretical models and empirical tests using birds and mammals. Annu.Rev. Ecol. Syst. 21:449-480.

Koerner, L. E. W. 1997. Demography and spatial analysis of salt marsh harvest mice (*Reithrodontomys raviventris*) inhabiting suboptimal habitat on San Pablo Bay. MS Thesis University of California, Davis.

Landis, W. G., R. A. Matthews, and G. B. Matthews. 1996. The layered and historical nature of ecological systems and the risk assessment of pesticides. Environ. Toxicol. Chem. 15:432-440.

May, B., T.A. Gavin, P.W. Sherman, and T.M. Korves. 1997. Characterization of microsatellite loci in the Northern Idaho ground squirrel, *Spermophilus brunneus* Mol. Ecol. 6:399-400.

Pitcher, E. J. and B. L. Keller. 1979. Aspects of interspecific interaction between *Reithrodontomys megalotis* and *Microtus montanus* in one acre enclosures. Res. Popul. Ecol. 21:81-104.

Shellhammer, H. S. 1982. *Reithrodontomys raviventris*. Mammal. Spec. 169:1-3

Shellhammer, H. S. 1989. Salt marsh harvest mice, urban development, and rising sea levels. Conserv. Biol. 3:59-65.

Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. Van de Lee, M. Hornes, A. Frijters, J. Pot, J. Peleman, M. Kuiper, and M. Zabeau. 1995. AFLP: a new technique for DNA fingerprinting. Nucl. Acids. Res. 23:4407-4414.

Costs and Schedule to Implement Proposed Project
Budget 10/01/97 - 09/30/97

	Year One	Year Two[1]	Total
PERSONNEL			
B. May 2 mo. @ 100% @ $5000 /mo	$ 10,500.*	$ 11,025.**	$ 21,525.
M. Johnson - P.I. 2 mo. @ 100% @ $6350/mo	13,335.*	14,002.**	27,337.
Graduate Research Assistant 7.5 mo @ 50% academic, 100% summer	16,899.+	17,744.+	34,643.
Technician 7 mo/3.5 mo @ 100% @$2500/mo	17,875.++	9,581.^	27,456.
TOTAL SALARIES	**$58,609.**	**$52,352.**	**$110,961.**
Fringe Benefits *24%, **24.5%, +3.8%, ++23.72%, ^24.43%	10,603.	9,147.	19,750.
TOTAL PERSONNEL	**$69,212.**	**$61,499.**	**$130,711.**
Equipment (Thermocycler, traps, scales)	10,700.	0.	$10,700.
Student Fees ($1,495.per qtr. 1st year) ($1,569.per qtr. 2nd year)	4,485.	4,709.	9,194.
Supplies (Bait, cotton, flags, eartags, lab. supplies)	9,000.	5,000.	14,000.
Travel (Domestic, field site)	7,100.	7,600.	14,700.
TOTAL DIRECT COST	**$100,497.**	**$78,808.**	**$179,305.**
INDIRECT COST @ 44.5% (Total Direct Costs Less Student Fees & Equipment)	37,964.	32,974.	70,398.
TOTAL COSTS	**$138,461.**	**$111,782.**	**$250,243.**

1. Personnel and fees are increased by 5% in the second year

Scheduled milestones

Year 1. The first two objectives will be completed.

1) Sample the entire northern range of both species to collect hair and/or cheek cell samples from SMHM and WHM in as many habitats as possible.

2) Using Amplified Fragment Length Polymorphisms (AFLP), develop a marker(s) to reliably distinguish between the two species.

Year 2. The remaining objectives will be completed.

3) Assess the amount of genetic variation throughout the North Bay, Suisun Bay, and Delta in the two species.

4) Determine if the northern populations are subdivided into isolated subpopulations.

5) Determine if any introgression is occurring between the SMHM and the WHM at any point along the interface of the two species.

6) Compare the demographic characteristics of the individuals captured at each location with the same characteristics of animals from established populations of SMHM to evaluate the potential viability of populations in the sample locations.

7) Evaluate samples submitted by the resources agencies to provide species identifications for monitoring projects.

Third Party Impacts

There will be no negative third party impacts. We will cooperate fully with federal and state agencies to make the genetic marker analysis available to any group conducting surveys or monitoring programs.

Applicant qualifications

Dr. Bernie May is an Associate Research Biologist and Director of the Genomic Variation Laboratory in the Department of Animal Science at the University of California at Davis. He received his PhD in Genetics from the Pennsylvania State University in 1980 and then spent 14 years at Cornell as Director of the Cornell Laboratory for Ecological and Evolutionary Genetics. During that time he collaborated on genetic studies of more than 100 diverse taxa. He came to Davis in 1995 and has continued to bring his genetics perspective to understanding the distribution of variation within and among populations. He has published over 90 scientific papers. Some of his recent molecular genetic work on the partitioning of genetic variation in the endangered Northern Idaho ground squirrel directly relates to this study.

Dr. Michael Johnson is an Associate Research Engineer in the Department of Civil and Environmental Engineering at the University of California at Davis, and is a Graduate Advisor for the Conservation Biology Area of Emphasis in the Graduate Group in Ecology. Trained as a small mammal population biologist, he has been conducting mark-recapture studies on small mammals for over 20 years and has numerous publications on the demography and dispersal. For the past 3 years, he has been studying the demography and ecology of salt marsh harvest mice and other species residing in the wetlands of the San Pablo Bay National Wildlife Refuge. He holds all appropriate federal and state permits to conduct the research.

Facilities available

The DNA analyses will be conducted at the Genomic Variation Laboratory (GVL) in the Department of Animal Science at the University of California at Davis, CA. The GVL is under the direction of Dr. May and consists of 1200 sq. ft. of standard molecular biological resources capable of screening genomic libraries, doing PCR, sequencing, and conducting electrophoresis of DNA and allozymes. It includes such equipment as three ultracold freezers, fume hood, numerous electrophoretic power supplies, gel dryer, incubation ovens, shakers, water baths, acrylamide, agarose, and starch gel boxes, microfuges, gel photographic and imaging systems, three thermocyclers, PC and MAC microcomputers and software, and other relevant laboratory equipment. The recent acquisition of a Molecular Dynamics 595 fluorimager has extended the capacity of our laboratory to gather larger quantities of data in a shorter period of time. This laboratory is located in a building which houses several additional molecular laboratories with which we share additional equipment (e.g., darkroom, nanopure water, autoclaves, etc.).

Compliance with Standard Terms and Conditions

Standard terms and conditions of this contract are those that apply to state agencies as shown in Table D-1 of the RFP. These do not require any paperwork until the final contract is in place. The standard University of California Office of Research Data Sheet has been completed and includes approval for submittal of this proposal by the Dean of the College of Engineering, and the UC Davis Office of Research. This approval includes agreement by Dr. Michael Johnson to the standard terms and conditions of the UC Davis OR data sheet. These forms are attached.

Item 8

NONDISCRIMINATION COMPLIANCE STATEMENT

COMPANY NAME: THE REGENTS OF THE UNIVERSITY OF CALIFORNIA

The company named above (hereinafter referred to as "prospective contractor") hereby certifies, unless specifically exempted, compliance with Government Code Section 12990 (a-f) and California Code of Regulations, Title 2, Division 4, Chapter 5 in matters relating to reporting requirements and the development, implementation and maintenance of a Nondiscrimination Program. Prospective contractor agrees not to unlawfully discriminate, harass or allow harassment against any employee or applicant for employment because of sex, race, color, ancestry, religious creed, national origin, disability (including HIV and AIDS), medical condition (cancer), age, marital status, denial of family and medical care leave and denial of pregnancy disability leave.

CERTIFICATION

I, the official named below, hereby swear that I am duly authorized to legally bind the prospective contractor to the above described certification. I am fully aware that this certification, executed on the date and in the county below, is made under penalty of perjury under the laws of the State of California.

OFFICIAL'S NAME: Sandra M. Dowdy
Contracts and Grants Analyst

DATE EXECUTED: JUL 22 1997

EXECUTED IN THE COUNTY OF: Yolo

PROSPECTIVE CONTRACTOR'S SIGNATURE: Sandra M. Dowdy

PROSPECTIVE CONTRACTOR'S TITLE:

PROSPECTIVE CONTRACTOR'S LEGAL BUSINESS NAME: THE REGENTS OF THE UNIVERSITY OF CALIFORNIA